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# Electron Microscope Study of the Infection Process of Blue-Green Algae Virus

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**Electron Microscope Study of the Infection**

**Process of Blue-Green Algae Virus**

**by**

**Thomas A. Rudd**

**A Thesis Submitted to the Faculty of the Graduate School  
of Loyola University in Partial Fulfillment  
of the Requirements for the Degree of  
Master of Science**

**June 1972**

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## INTRODUCTION

Algae, commonly known as pond scums and seaweeds, are among the simplest forms in the plant kingdom. In recent years algae have begun to receive the in-depth attention which has emphasized the widespread importance of such plants in the possible solution of a variety of contemporary problems. For example, it seems feasible at the present time that algae might well be used in solving the world food problem. Chlorella (unicellular green alga), for instance, is one form of algae which has a high nutritional value based on the high protein content in amounts comparable to the proportions of protein found in higher plants (Schwimmer and Schwimmer, 1955). It has been established by experimentation that a quarter pound of Chlorella will supply the human daily minimal vitamin requirements, except for ascorbic acid (Burlew, 1953). However, it must be born in mind that more extensive investigations must be made before algal food is made practical, both in terms of production and palatability.

Importance of algae is generally recognized because of their suitability as basic research material in the field of plant pathology. Cyanophyta, for example, can be easily obtained and cultured and can be studied at the molecular level. Recently it has been found that plants in Cyanophyta are subject to viral diseases. Safferman and Morris (1963) isolated the blue-green algae virus LPP-1. It is believed that basic research dealing with such algae viruses may lead to extremely valuable knowledge concerning the pathogenic processes which may eventually be applied to combat some of the higher plant diseases responsible for the annual destruction of millions of dollars worth of crops and plants of commercial value.

The present study will investigate the processes through which blue-green algae virus LPP-1 invades the host cell and reproduces itself.

## REVIEW OF LITERATURE

So far we do not know of any plant virus that can enter a plant without physical injury to the susceptible host. The injury to the plant cell has to be mild enough so that the cell may not be killed. Wildman (1959) stated that the union of the virus with the host cell is instantaneous which is evidenced by no reduction in infection by washing the tissue immediately after inoculation or by inoculating N. glutinosa leaves with TMV-RNA and then treating with RNase. The union is the first event which occurs after inoculation and this process is irreversible.

Franklin et al (1959) found that TMV is composed of 2200 identical protein units arranged in a helix enclosing a single strand of RNA. It is believed that RNA, while tightly enclosed in the protein coat, cannot carry out its function. The protein coat of TMV is not infectious, whereas it is well known that RNA of the virus is infectious. The mechanism involved in stripping off of the protein coat of TMV in living cells is not yet known. Hausers-Casterman and Jeener (1957) showed that if mosaic inoculated leaves are infiltrated with RNase within two hours after inoculation virus will not multiply. If RNase treatment is given inoculated leaves after two hours of inoculation the liberated RNA will become protected with TMV protein or with subcellular components. This indicates that two hours are needed for the RNA to be coated with protein.

It is believed that once the infective RNA enters the infection site of the cell, resistance to ultraviolet irradiation increases. Siegal et al (1957) using ultraviolet techniques, demonstrated that it required two and a half to five hours to free the RNA from the protein coat and make the necessary contacts with the infection site. Fraenkel-Conrat et al (1958) and Kassanis (1960) found that local lesions appeared earlier when local lesion hosts were inoculated with RNA than with the whole TMV. Engler and Schramm (1959) also showed that the

latent period was 10 hours less with RNA than with complete virus.

It is known that in normal cells RNA synthesis is directed by nuclear DNA. There is no evidence that viral RNA is so directed. Bald and Salberg (1961) reported the abnormal activity in or around the nuclei of TMV infected cells. On the contrary, Schramm and Rottger (1959), by using fluorescent TMV antibodies on infected tissue sections, reported that virus protein was synthesized in the cytoplasm and that no virus protein was formed in the nucleus or the chloroplasts.

Gierer (1957) studied the stepwise degradation of TMV-RNA. He stated that the entire 6400 nucleotide chain must be preserved in order to demonstrate infectivity. Cochran and Dhaliwal (1962), Kim and Wildman (1962), Karasek and Schramm (1962), and Cornuet and Astier-Manifacier (1962) have reported the synthesis of TMV-RNA in cell free systems. All the above mentioned four groups used one to twenty day old TMV infected tissue as a source of polymerase, TMV-RNA, primers, nucleotide triphosphates (ATP, CTP, GTP, and UTP) and RNase inhibitors (bentonite and polymethacrylic acid) in their reaction mixtures. They reported their data on the basis of increase in infectivity of local lesion forming hosts such as Phaseolus vulgaris varieties pinto and scotia, Nicotiana glutinosa, N. tabacum variety xanthi. The rise in infectivity from 0 to 4 fold was recorded.

#### BLUE-GREEN ALGAE VIRUS

Blue-green algae virus LPP-1 was first isolated by Safferman and Morris in 1963. They also studied the growth characteristics of BGA virus (1964). They stated that BGA virus strain LPP-1 formed two distinct plaque variants. During subsequent propagation each of the isolated variants eventually reverted to a mixture of both plaque types. The large plaque isomer, multiplying at a somewhat faster rate showed maximal virus production within 60 hours after

infection; maximal titer of a small plaque mutant was reached after 76 hours inoculation period. Singh and Singh (1967) isolated the first phycovirus strain LPP-1 and propagated it on the BGA Plectonema boryanum. Five strains of cyanophages have been isolated from local polluted pond waters.

Safferman et al (1969) described the purification of a new BGA virus SM-1 which infects only unicellular forms. This virus is a polyhedron with no obvious tail and with an average diameter of about 88 m $\mu$ . They indicated that this virus was distinct from the BGA virus LPP-1. In 1967 Safferman and Morris observed phycovirus populations in eleven of the twelve waste stabilization ponds studied. These populations were comprised solely of BGA viruses. Two virus types were observed, one of which was related to the previously reported LPP-1 virus. Padan et al (1967) also isolated viruses active against Plectonema boryanum from fresh water ponds in Israel. On the basis of host specificity, DNA content, and fine structure one of the viruses appeared to be related to the virus studied by Safferman and Morris (1963, 1964a).

The morphology of Safferman's BGA virus has been studied by electron microscopy and physicochemical methods (Luftig and Haselkorn, 1967). The virion has a short forked tail (100 to 200 Å long, 150 Å in diameter), an outer sheath, an inner core and a capsid attached to one of the vertices of the polyhedral head. The head capsid edge to edge distance is 600 Å, based upon internal calibration of the magnification in electron micrographs by use of line-line spacing of catalase crystals. Luftig and Haselkorn (1968) also indicated that BGA virus LPP-1 had a head capsid hexagonal in projection with edge to edge distance of  $600 \pm 20$  Å, and a short tail 200 Å long and 150 Å in diameter attached to one of the capsid vertices.

Goldstein et al (1967) indicated that adsorption and one step growth experiments performed with BGA virus LPP-1 under normal conditions revealed

a slow adsorption rate, in comparison with bacteriophage systems, followed by a 7-hour latent period. The burst size was approximately 100 virus particles per cell. They found that molecular weight of the virus obtained from sedimentation and diffusion measurements was  $51 \pm 3 \times 10^6$  daltons, and the hydration was calculated to be 0.37 grams of water per gram of virus.

Brown et al (1966) studied the mode of infection of BGA Plectonema boryanum by BGA virus. The virus attaches itself to the cell wall and the DNA is then injected into the cell after the manner of some bacteriophages. They suggested that DNA of the virus is formed in the nucleoplasm where it migrates to the photosynthetic lamellae. Smith et al (1966a) described that after infection of algae by BGA virus, photosynthetic lamellae invaginated. The virus particles were seen in contact with the cell wall, and inside the cell attached to the photosynthetic lamellae. Here they remained attached after the cells were completely lysed. Smith et al (1966b) showed that particles of BGA virus attach to the walls of the host cells by a long tail, like that of some bacteriophages, and at infection they inject their nucleic acid into the cells in a bacteriophage-like manner. In cultures, inoculated some days earlier, masses of virus particles were found in thick sections of cells of Plectonema boryanum, P. Notatum, and Lyngbya sp. Smith et al (1967) also studied the progress of lysis along the filaments of virus infected algae, Plectonema boryanum followed by means of time lapse photography using phase optics. The period of time required to lyse cells is approximately 45 seconds.

## MATERIALS AND METHODS

### PREPARATION AND GROWTH OF ALGA:

This study involved two strains of Cyanophyta: Plectonema boryanum strain 594 and Lyngbya species strain 487. The strains were grown in Bold's Basal Medium prepared according to the following method: six stock solutions, 400 ml in volume, were employed, each containing one of the following salts in the concentration listed:

NaNO <sub>3</sub> . . . . .	10gm
CaCl <sub>2</sub> ·2H <sub>2</sub> O. . . . .	1gm
K <sub>2</sub> HPO <sub>4</sub> . . . . .	3gm
KH <sub>2</sub> PO <sub>4</sub> . . . . .	7gm
MgSO <sub>4</sub> ·7H <sub>2</sub> O. . . . .	3gm
NaCl. . . . .	1gm

For the dilution, 10 ml of each stock solution was added to 940 ml of pyrex distilled H<sub>2</sub>O. One ml of each of four stock trace element solutions prepared as follows was added to the above solution:

I. EDTA Stock Solution

EDTA . . . . .	50gm
KOH . . . . .	31gm
Distilled Water. . . . .	1 liter

II. Iron Stock Solution

FeSO <sub>4</sub> ·7H <sub>2</sub> O . . . . .	4.98gm
Acidified Water. . . . .	1 liter

III. Boron Stock Solution

H <sub>3</sub> BO <sub>3</sub> . . . . .	11.42gm
Distilled Water. . . . .	1 liter

Then 100 ml of Bold's media was placed in 300 ml bell shaped culture flasks and covered with a metal lid. The flasks, with media, were autoclaved at 240°C for 15 minutes. The flasks were allowed to cool under sterile conditions and 2.5 ml of two to three week old alga were transferred into each flask. Six flasks of each species (Plectonema and Lyngbya) were prepared this way. The flasks were then transferred to an environmental growth chamber with 21°C temperature and 200 foot-candle light intensity from "cool white" fluorescent lamps. The strains of alga were then cultured for three weeks.

## METHOD OF INFECTION

At the end of three weeks of growth each strain of blue-green algae was infected with blue-green algae virus LPP-1 (prepared by the isolation method of Dhaliwal, 1968). The inoculation procedure was as follows: 1 ml of concentrated virus with  $10^8$  plaque forming units was added to each flask of three week old cultured algae and mixed slowly. At five minute intervals 15-20 ml of algae with virus were drawn from the flask and centrifuged (refrigerated Sorvall model RC2-B) at 2000 RPM for five minutes. The supernatant was then poured off and the pellet was used for E.M. studies.

## FIXING AND STAINING

The pellets obtained above were fixed for one hour at room temperature in a mixture of Cacodylate buffer (0.1M cacodylate solution: 21.4gm/l: pH adjusted with NaOH to 7.2-7.4) containing 3% acrolein and 3% glutaraldehyde. After one hour in this fixative the solution was centrifuged at 2000 RPM for ten minutes. The supernatant was poured off and the pellet was next fixed in 2% osmium tetroxide for one hour at room temperature. The sample was again centrifuged at 2000 RPM for ten minutes. The supernatant was poured off and the pellet stained in 0.5% uranyl acetate overnight at 5°C. This sample was then centrifuged at 2000 RPM for ten minutes.

## DEHYDRATION

The pellet obtained above was dehydrated in the following manner:

1. 50% ethyl alcohol for thirty minutes.
2. 70% ethyl alcohol for fifteen minutes.
3. 80% ethyl alcohol for fifteen minutes.
4. 95% ethyl alcohol for fifteen minutes with one repetition.
5. 100% ethyl alcohol for fifteen minutes.
6. Propylene oxide for fifteen minutes.

## PRE-EMBEDDING PROCEDURE

The embedding medium consisted of an one to one proportion of propylene oxide and epoxy resin mixture (20 parts Araldite #502, 20 parts Epon #812 and 60 parts of dodecenyl succinic anhydride) with 0.2 ml of DMP-30 added for every 10 ml of the epoxy-propylene oxide mixture. The pellets were left in this mixture overnight at room temperature. The pellets were covered loosely so that the propylene oxide was allowed to evaporate.

## FINAL EMBEDDING PROCEDURE

The pellets embedded in epoxy-propylene oxide were poured onto the filter paper to separate them from the pre-embedding mixture. The pellets were transferred into the plastic capsules containing epoxy resin. The latter was prepared by adding 0.2 ml of DMP-30 per 10 ml of epoxy resin. The capsules were placed in a 37°C oven for five to six hours, and a 62°C oven for 48 hours. Finally, the capsules were removed from the oven and allowed to cool for trimming and sectioning.

## SECTIONING AND STAINING

Sections were cut from the trimmed blocks using glass knives (prepared with a LKB 7800-B knife maker) with a Sorvall Porter Blum ultramicrotome MT-1. The sections were collected in troughs containing water. Only the gray sections were picked up on 300 mesh copper grids and stained.

## STAINING

The sections were first stained in uranyl acetate for ten to thirty minutes. Sections were washed in glass distilled water and counter stained in lead citrate for ten to thirty minutes.

The staining solutions were prepared as follows:

### Uranyl Acetate

$\text{UO}_2(\text{C}_2\text{H}_3\text{O}_2)_2 \cdot \text{H}_2\text{O}$ . . . . .	8.0 gm
Glass distilled water . . . . .	100 ml



**Lead Citrate**

$\text{Pb}(\text{NO}_3)_2$	1.33gm
$\text{Na}_3\text{C}_6\text{H}_5 \cdot 2\text{H}_2\text{O}$	1.76gm
1N NaOH.	8ml
Glass distilled water.	.50ml

Stained sections were studied and electron micrographs were taken with a Hitachi HU-11 electron microscope.

## RESULTS AND DISCUSSION

The preliminary account of the infection process of the BGA virus was given by Smith et al (1966). They showed that the most characteristic sign of infection was the lateral displacement of the photosynthetic lamellae. Newly formed virus was closely associated with the lamellae. However, the time at which infection starts, and the mode of entry into the cell, whether the injury to the host tissue is required for the entry or not, was not elucidated.

### ELECTRON MICROSCOPE STUDIES OF PLECTONEMA BORYANUM

#### ELECTRON MICROSCOPE STUDIES AT 5 AND 10 MINUTES AFTER INFECTION OF PLECTONEMA BORYANUM WITH BLUE-GREEN ALGAE VIRUS

Figure 1 shows a healthy and an infected cell. This means that the infection of the algae with BGA virus started at five minutes after inoculation, or earlier. It was found that most of the filaments at this time were healthy and only a few cells were infected.

In figure 1 the left cell is completely lysed where only photosynthetic lamellae are seen. The cell to the right is healthy. Figure 2 shows a cell where infection has just started, indicated by the dark bodies in the center of the cell called virogenic stroma. Virogenic stroma usually develop in the nuclear part of the cell.

Figure 3 shows the healthy filament of the alga Plectonema boryanum at ten minutes after infection with BGA virus. On the other hand a similar filament is shown in figure 4 which is almost lysed. It is clear from figure 4 that the nucleoplasm is completely destroyed and virus particles appear as dark bodies in close association with photosynthetic lamellae.

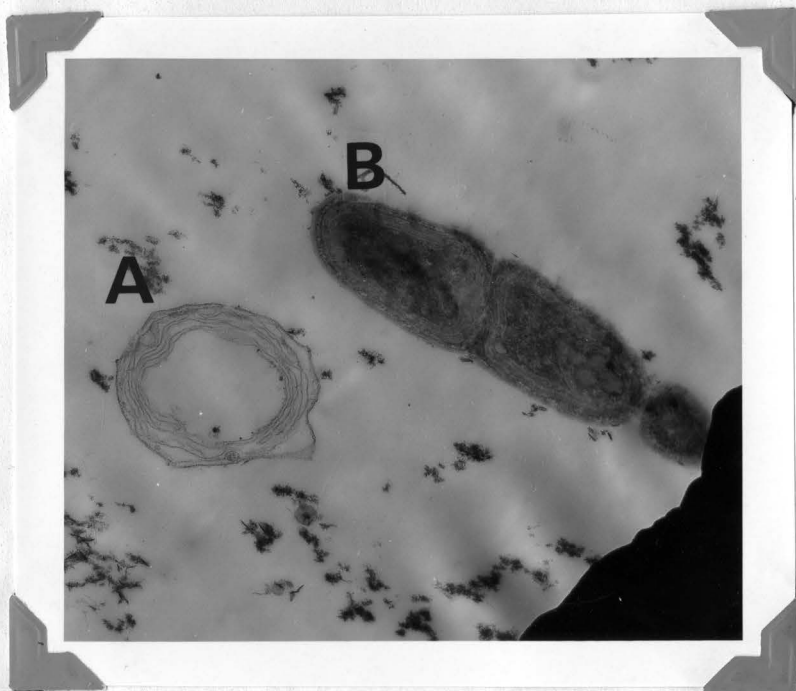


Fig. 1. Electron micrograph of Plectonema boryanum at 5 minutes after infection with blue-green algae virus. A, healthy. B, lysed. Magnification X 65,000.

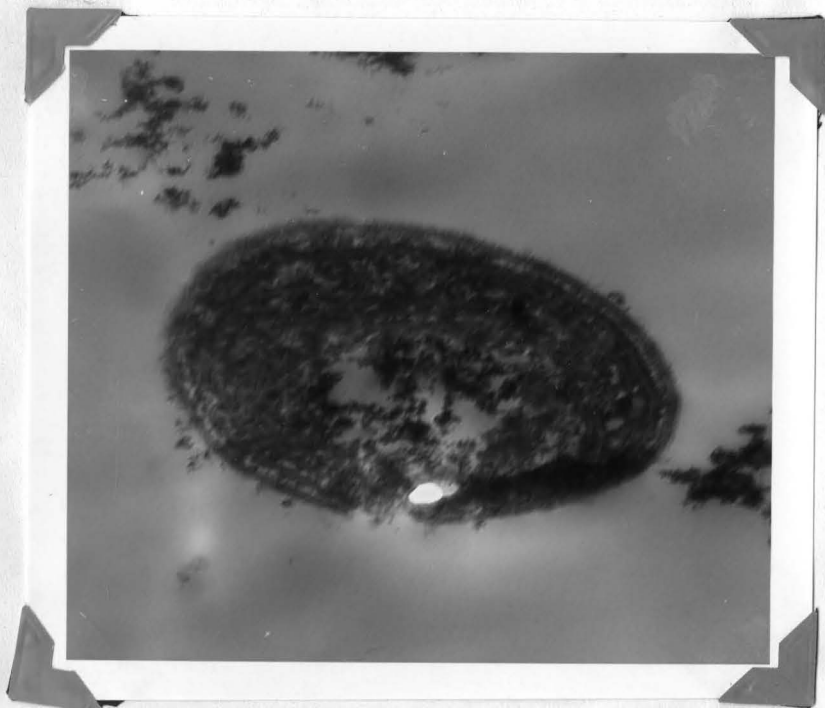


Fig. 2. Electron micrograph of *Plectonema boryanum* at 5 minutes after infection with blue-green algae virus. Magnification X 91,000.



Fig. 3. Electron micrograph of Plectonema boryanum at 10 minutes after infection with blue-green algae virus. Healthy filament. Magnification X 60,000.

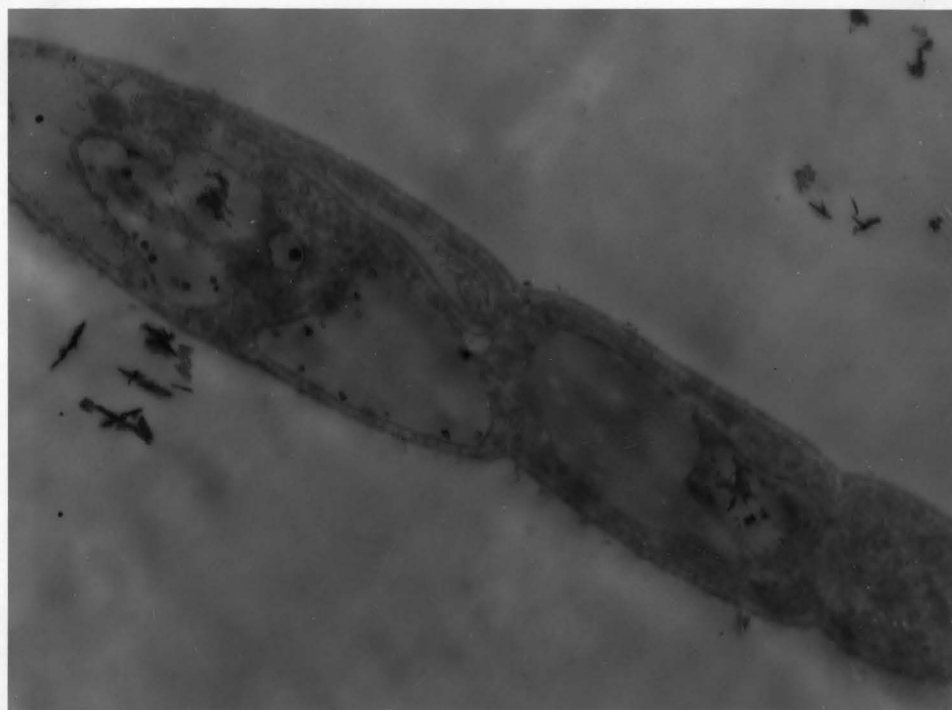


Fig. 4. Electron micrograph of Plectonema boryanum at 10 minutes after infection with blue-green algae virus. Infected filament. Magnification X 77,000.

ELECTRON MICROSCOPE STUDIES AT 15 AND 20 MINUTES AFTER INFECTION OF PLECTONEMA BORYANUM WITH BLUE-GREEN ALGAE VIRUS

Figure 5 indicates a healthy cell with virus attached to the cell wall by their long tails. This is just the beginning of infection process. Within a matter of seconds the cell will be lysed. It has been suggested by Smith et al (1966) that the injection of the DNA of the virus is similar to the bacteriophages. To the right is a cross section of the healthy filament. At 15 minutes after infection there are cells which are completely lysed as shown in figure 6. The nucleoplasm is all lysed and the virus particles as indicated by the dark spots are associated with the photosynthetic lamellae. Material at 20 minutes after infection was not processed.

ELECTRON MICROSCOPE STUDIES AT 25 AND 30 MINUTES AFTER INFECTION OF PLECTONEMA BORYANUM WITH BLUE-GREEN ALGAE VIRUS

Figure 7 shows an infected algal filament with new virus being synthesized in the nucleoplasm of the cell. Photosynthetic lamellae in these cells are being pushed away from their original positions. A completely lysed cell with displaced photosynthetic lamellae and virogenic stroma is seen in figure 8. It appeared from the studies made at 30 minutes after infection that hardly any algal cells were found which were not infected with virus. The material at 25 minutes after infection with BGA virus was processed but the capsules were spoiled during the preparation process.

ELECTRON MICROSCOPE STUDIES AT 35, 40, 45, 50, 55 AND 60 MINUTES AFTER INFECTION OF PLECTONEMA BORYANUM WITH BLUE-GREEN ALGAE VIRUS

Figures 9 through 17 show cells which are completely lysed. What is quite apparent is the thorough displacement and in some cases destruction of the photosynthetic lamellae. Figures 9 and 10 are examples of cells with destroyed lamellae.

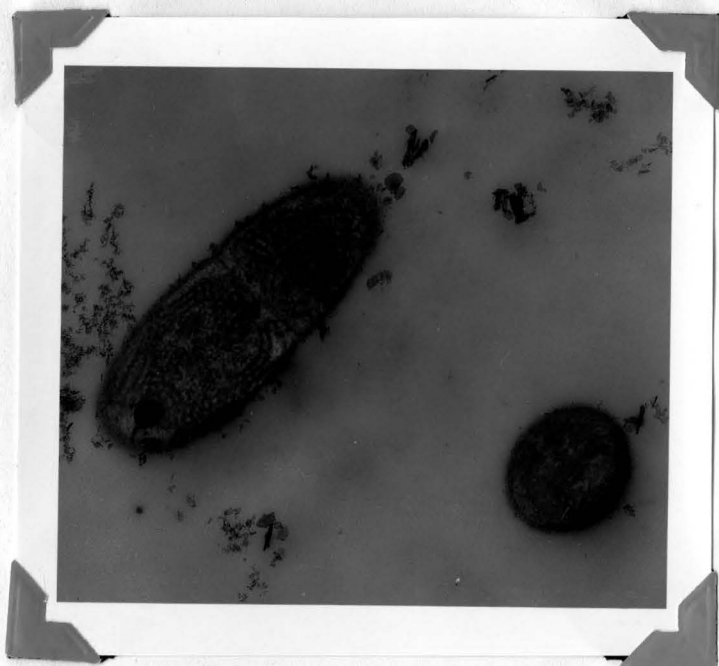


Fig. 5. Electron micrograph of Plectonema boryanum at 15 minutes after infection with blue-green algae virus. Healthy cells. Magnification X 60,000.



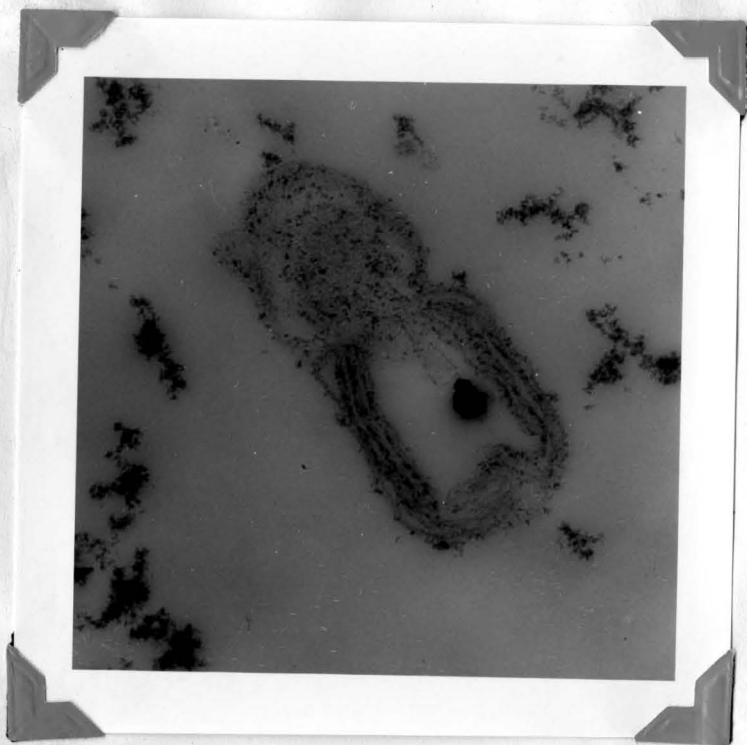


Fig. 6. Electron micrograph of Plectonema boryanum at 10 minutes after infection with blue-green algae virus. Lysed cell. Magnification X 100,000.



Fig. 7. Electron micrograph of *Plectonema boryanum* at 30 minutes after infection with blue-green algae virus. Infected cell. Magnification X 120,000.

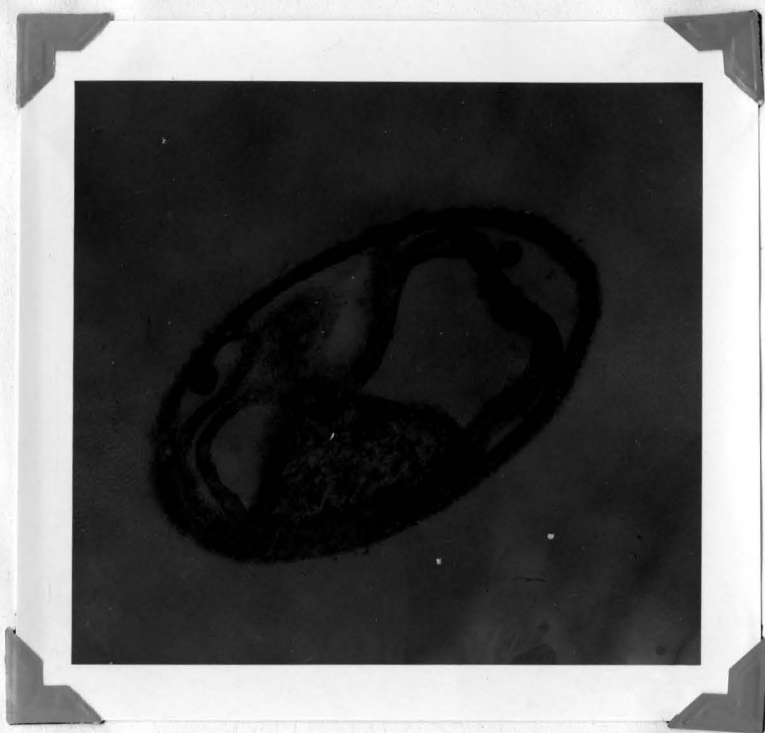


Fig. 8. Electron micrograph of Plectonema boryanum at 30 minutes after infection with blue-green algae virus. Lysed filament. Magnification X 91,000.

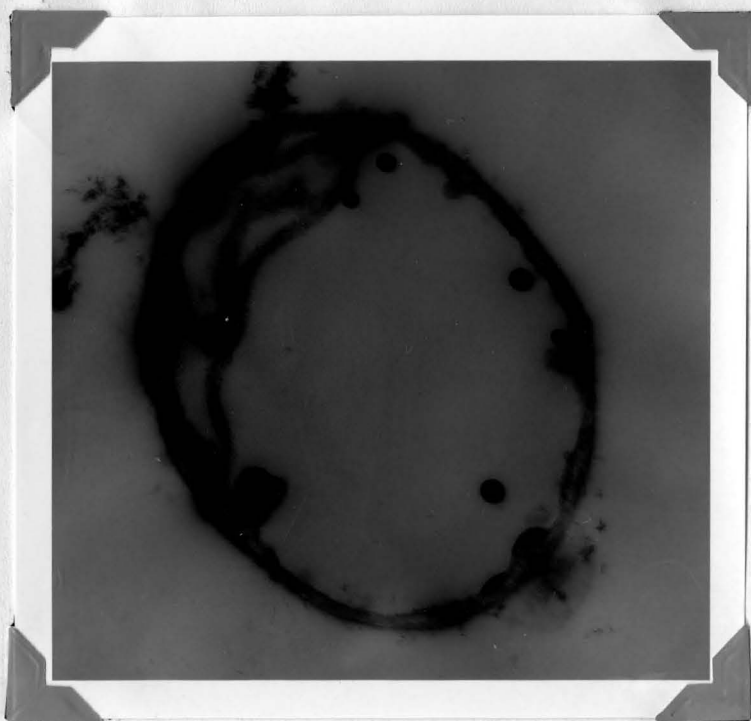


Fig. 9. Electron micrograph of Plectonema boryanum at 35 minutes after infection with blue-green algae virus. Lysed cell. Magnification X 131,000.

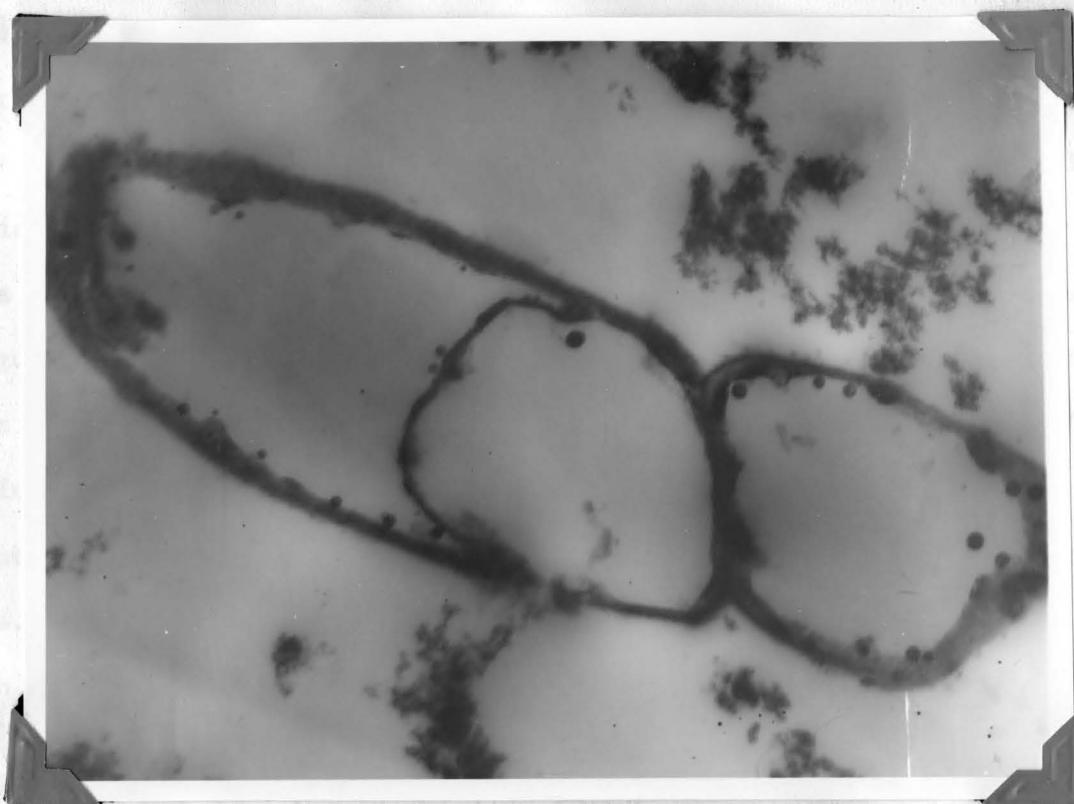


Fig. 10. Electron micrograph of *Plectonema boryanum* at 35 minutes after infection with blue-green algae virus. Lysed cells. Magnification X 102,000.

It is interesting to note that in most of these pictures the filaments are only one to three cells long. This is a result of total lysis: as lysis occurs the filaments are fragmented into smaller units so that eventually only scattered cells are seen.

With the lysis process well under way virus particles also become quite prevalent as illustrated in figures 13 and 14. The photosynthetic lamellae are noticeably bent with many virus particles spread throughout the cell.

Figure 12 is an interesting specimen in that one may see two processes occurring. In the upper right half of the filament a lysed cell is quite apparent with a few virus particles also evident. Just to the left of this lysed cell is another cell seen in an earlier stage of infection. Here virus particles are seen clustered in the nucleoplasm. Note that the lamella has not undergone complete displacement yet. Proceeding to the left, again in figure 12, is seen a lysed cell and to its left another cell undergoing infection.

In general, it is observed (figures 9 through 17) that as the time interval increases from 35 to 60 minutes after infection, complete lysis of cells occurs. These lysed filaments are usually only one, two or three cells in length with the majority of filaments being only one cell long.

#### ELECTRON MICROSCOPE STUDIES OF LYNGBYA SPECIES

#### ELECTRON MICROSCOPE STUDY OF LYNGBYA SPECIES AT 5, 10, 15, AND 20 MINUTES AFTER INFECTION WITH BLUE-GREEN ALGAE VIRUS

Lyngbya species and Plectonema boryanum both belong to Cyanophyta (blue-green algae). At the electron microscope level the morphological difference is in the cell size and in the filament as a whole. The cell size in Lyngbya species is smaller as compared to Plectonema boryanum. Furthermore, the tendency of the cells to remain together in long chains is more prevalent in Lyngbya species than in Plectonema boryanum.



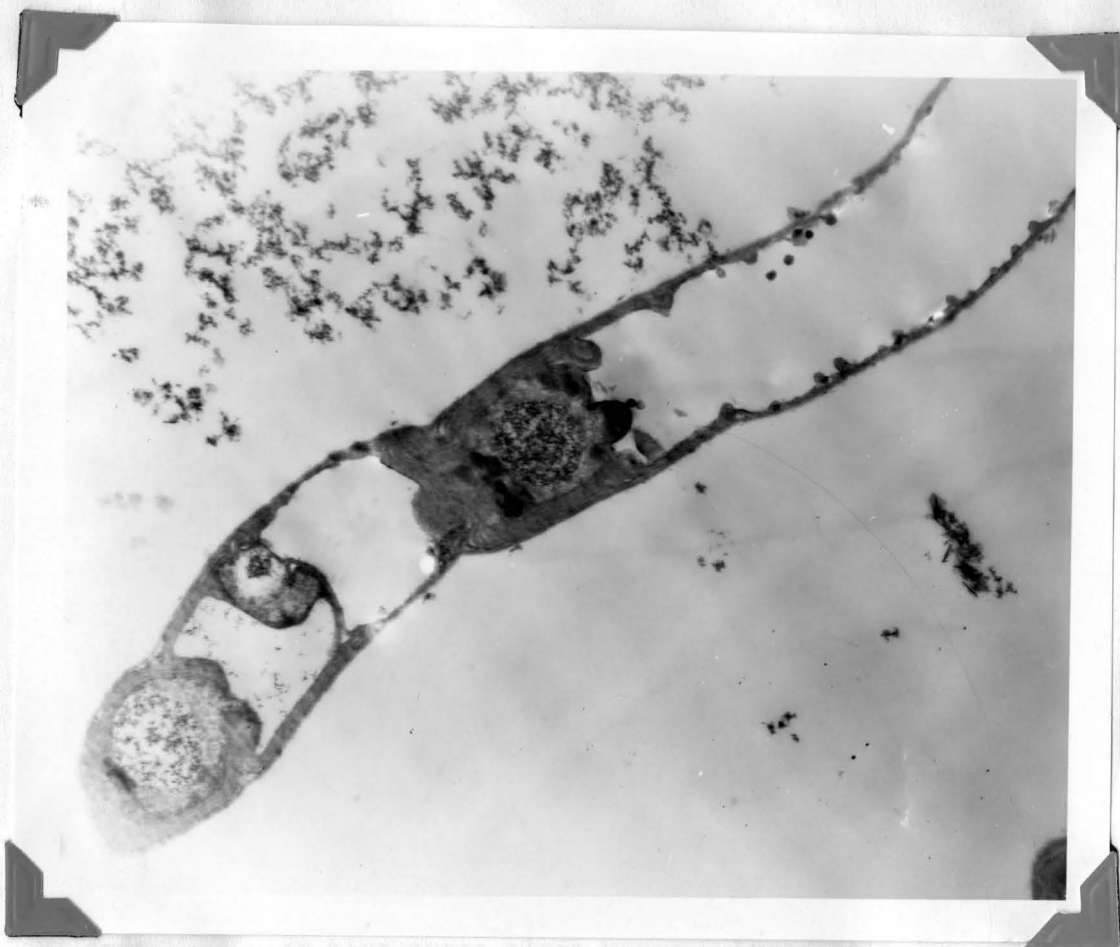


Fig. 11. Electron micrograph of *Plectonema boryanum* at 45 minutes after infection with blue-green algae virus. Lysed filament. Magnification X 27,000.



Fig. 12. Electron micrograph of Plectonema boryanum at 40 minutes after infection with blue-green algae virus. Lysed cells. Magnification X 128,000.



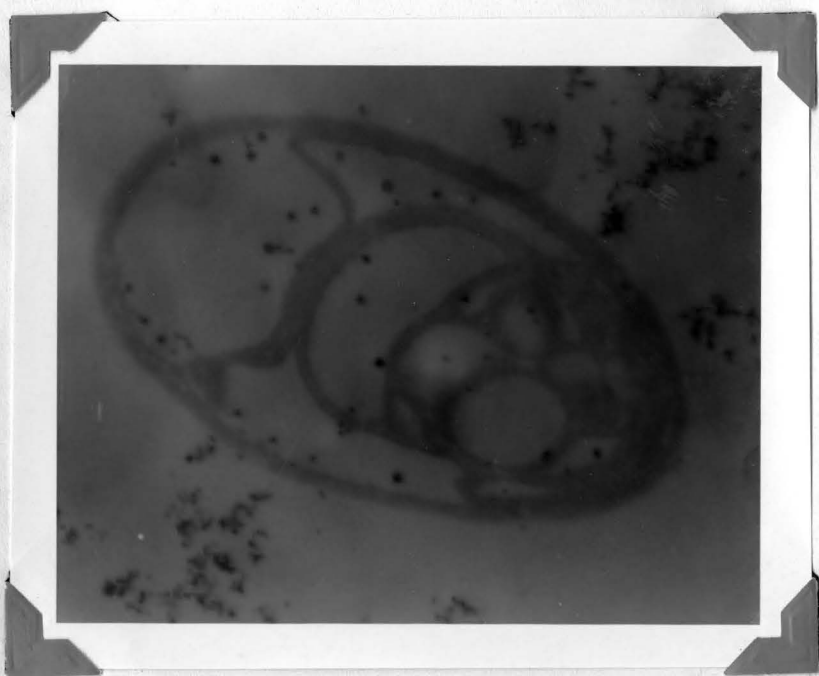


Fig. 13. Electron micrograph of Plectonema boryanum at 50 minutes after infection with blue-green algae virus. Lysed cell. Magnification X 163,000.

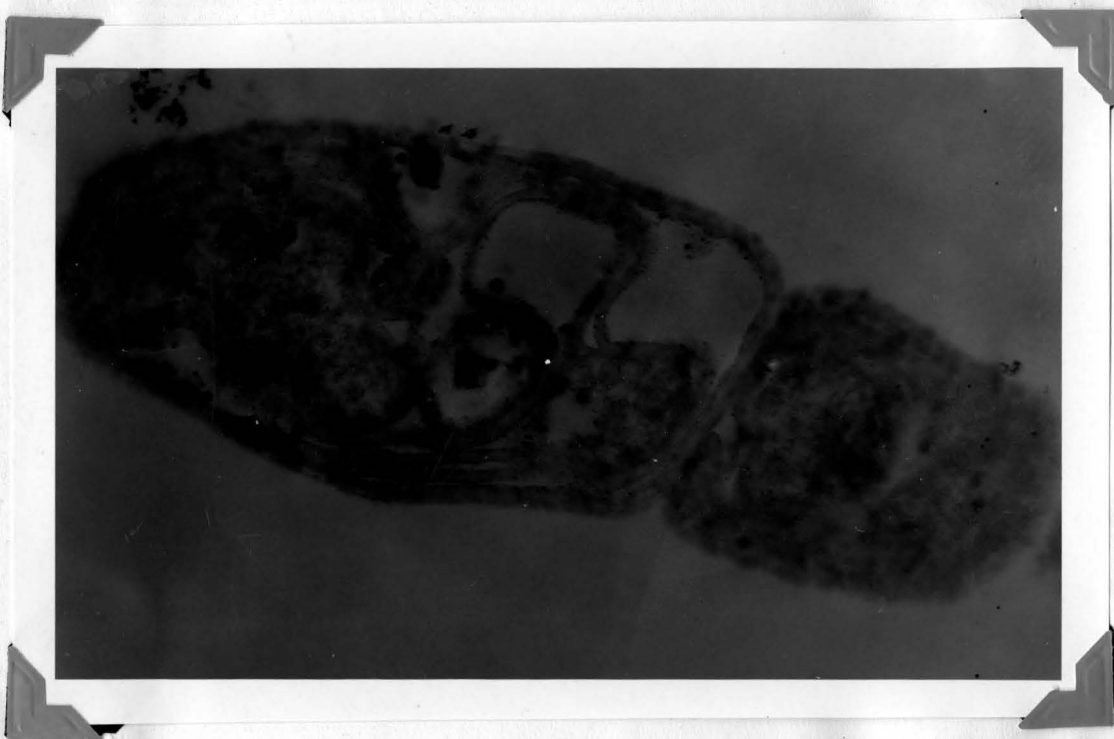


Fig. 14. Electron micrograph of Plectonema boryanum at 50 minutes after infection with blue-green algae virus. Lysed cells. Magnification X 168,000.

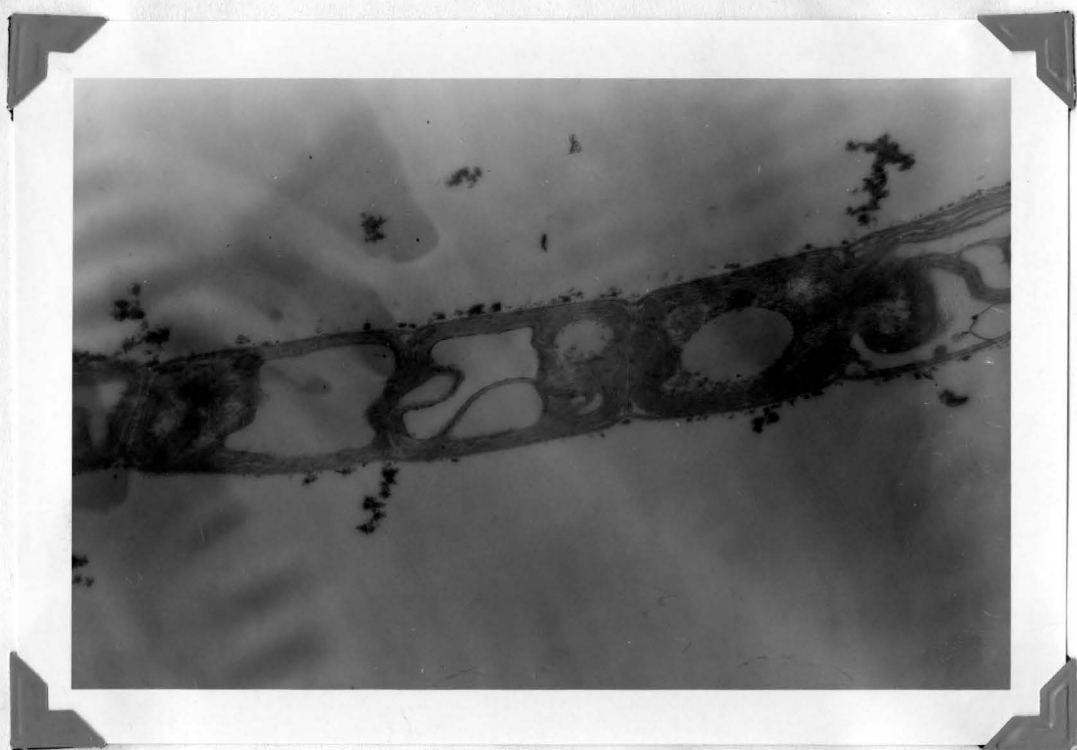


Fig. 15. Electron micrograph of *Plectonema boryanum* at 55 minutes after infection with blue-green algae virus. Lysed filament. Magnification X 140,000.

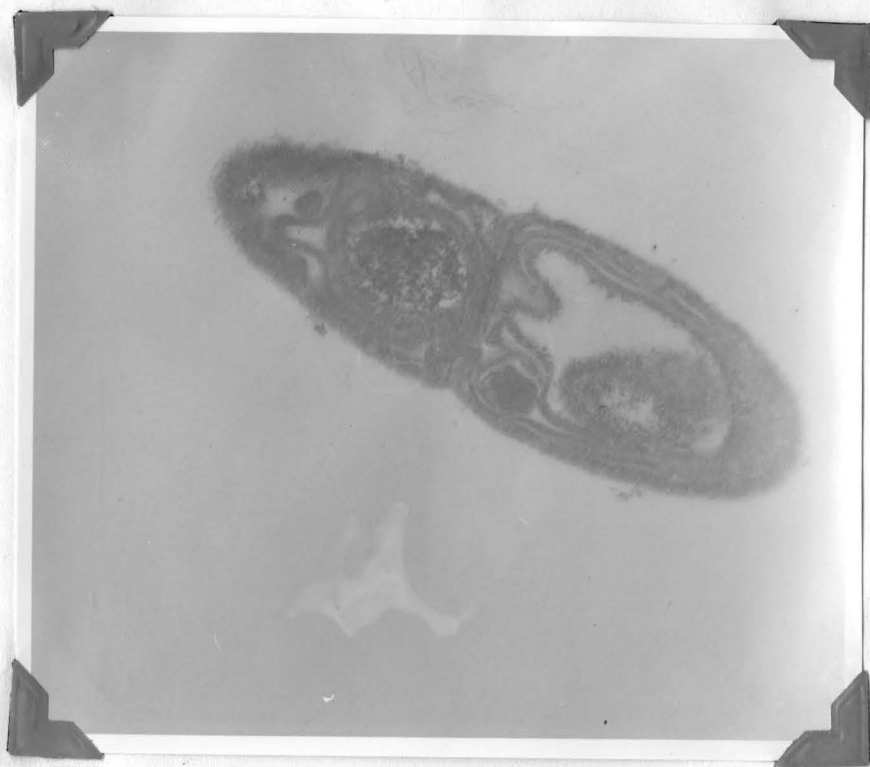


Fig. 16. Electron micrograph of *Plectonema boryanum* at 60 minutes after infection with blue-green algae virus. Lysed cells. Magnification X 51,000.

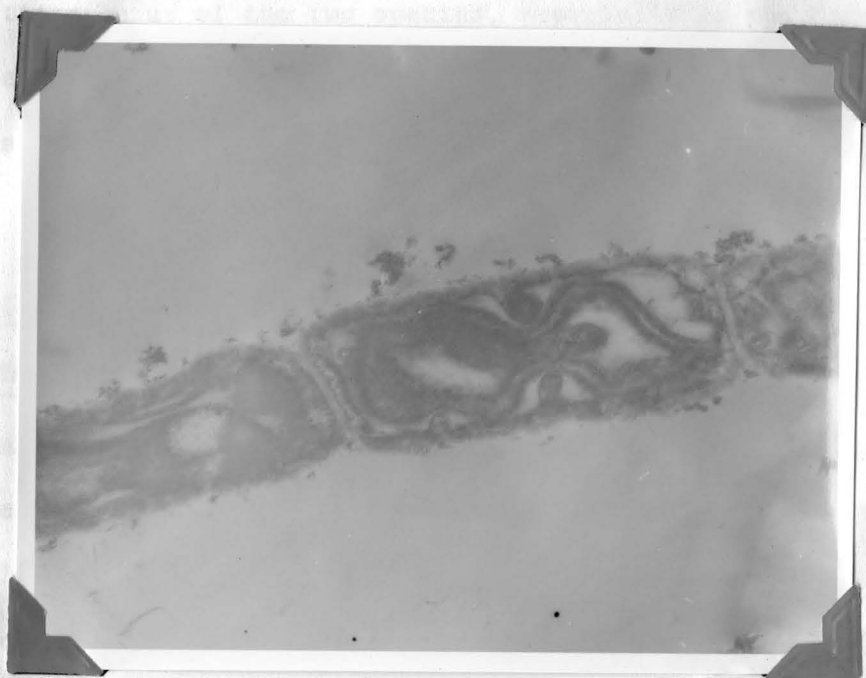


Fig. 17. Electron micrograph of Plectonema boryanum at 60 minutes after infection with blue-green algae virus. Lysed filament. Magnification X 68,000.

The most important characteristic by which both genera differ is in the time required for the infection process to start. As it is seen in figures 18, 19 and 20, there is no suggestion of infection in Lyngbya species at 5, 10 and 15 minutes. It appeared that infection started only at 20 minutes (fig. 21). During this period only a few completely lysed cells were found and the rest of the observed cells were healthy. Whereas in the case of Plectonema boryanum lysed cells were detected at 5 minutes after inoculation of algae with blue-green algae virus (fig. 1). This difference might be due to the genetic make up of the two species, especially in the formation of the outside sheath through which the virus makes its entry into the cell.

Newly synthesized virus can be seen associated with photosynthetic lamellae in a lysed cell in figure 21.

#### ELECTRON MICROSCOPE STUDY OF LYNGBYA SPECIES AT 25, 40, 50, 60, 70, and 80 MINUTES AFTER INFECTION WITH BLUE-GREEN ALGAE VIRUS

The examination of figures 22 through 26 reveals that the infection process has started as evidenced by the changes such as displacement of photosynthetic lamellae and the formation of virogenic stroma. But it appears that the rate at which new virus synthesis takes place in Lyngbya species is very slow. This is evidenced by the fact that most of the filaments appear healthy up to 70 minutes after infection with blue-green algae virus. However, the progressive changes are observed with infection time: virus particles are seen inside the cells (fig. 26) and the nucleoplasm appears to be destroyed (fig. 27). It appears from figure 27 that the photosynthetic lamellae are not easily displaced as a result of infection in the Lyngbya species. However, this change is very drastic in Plectonema boryanum.

At 80 minutes after infection most of the cells observed were lysed (fig. 28). The nucleoplasm and cell walls are destroyed and the newly formed virus

particles are found associated with the photosynthetic lamellae.



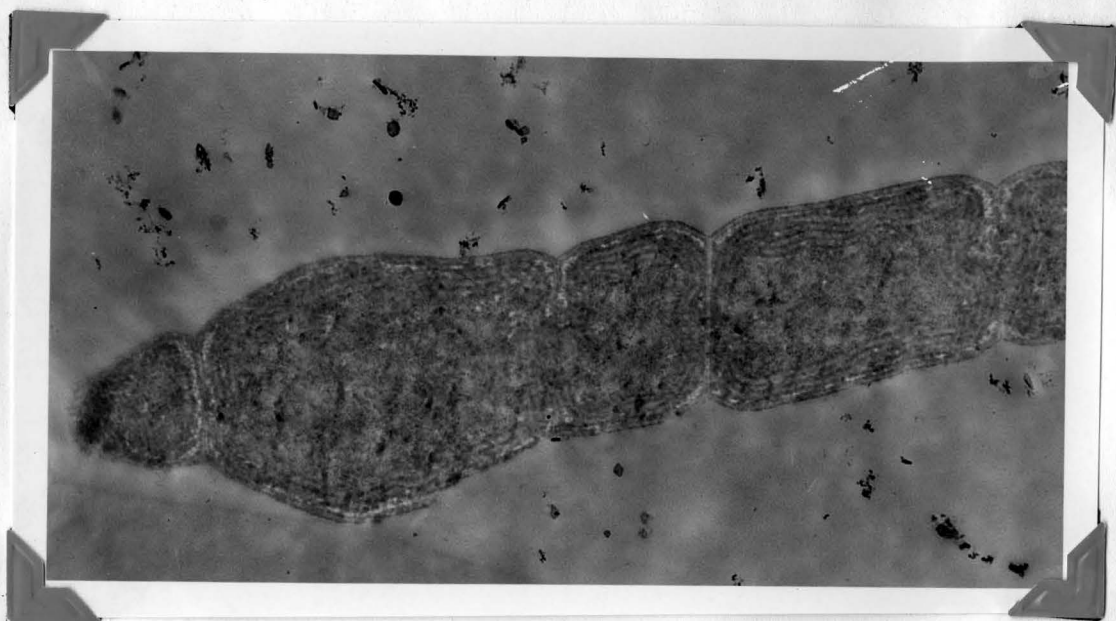


Fig. 18. Electron micrograph of Lyngbya species at 5 minutes after infection with blue-green algae virus. Healthy filament. Magnification X 80,000.



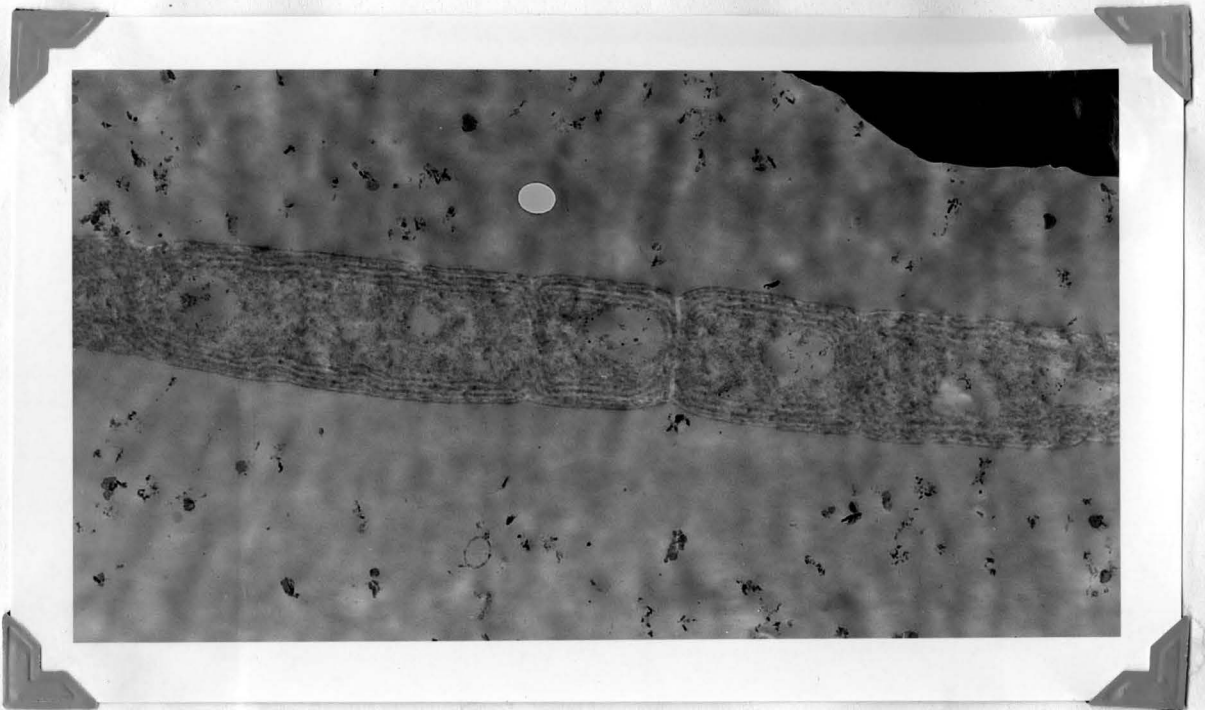


Fig. 19. Electron micrograph of Lyngbya species at 10 minutes after infection with blue-green algae virus. Healthy filament. Magnification X 63,000.



Fig. 20. Electron micrograph of Lyngbya species at 15 minutes after infection with blue-green algae virus. Healthy filament. Magnification X 128,000.

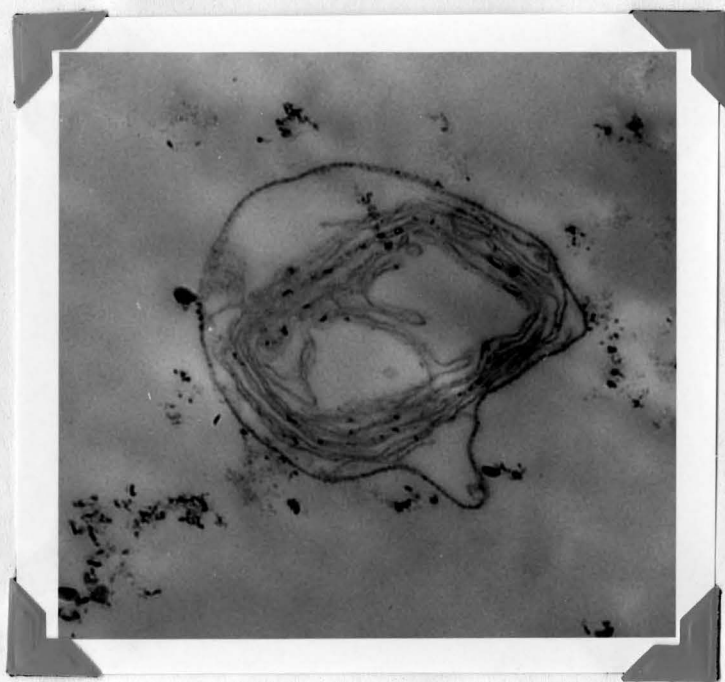


Fig. 21. Electron micrograph of Lyngbya species at 20 minutes after infection with blue-green algae virus. Lysed cell. Magnification X 100,000.



Fig. 22. Electron micrograph of Lyngbya species at 25 minutes after infection with blue-green algae virus. Healthy filament. Magnification X 100,000.

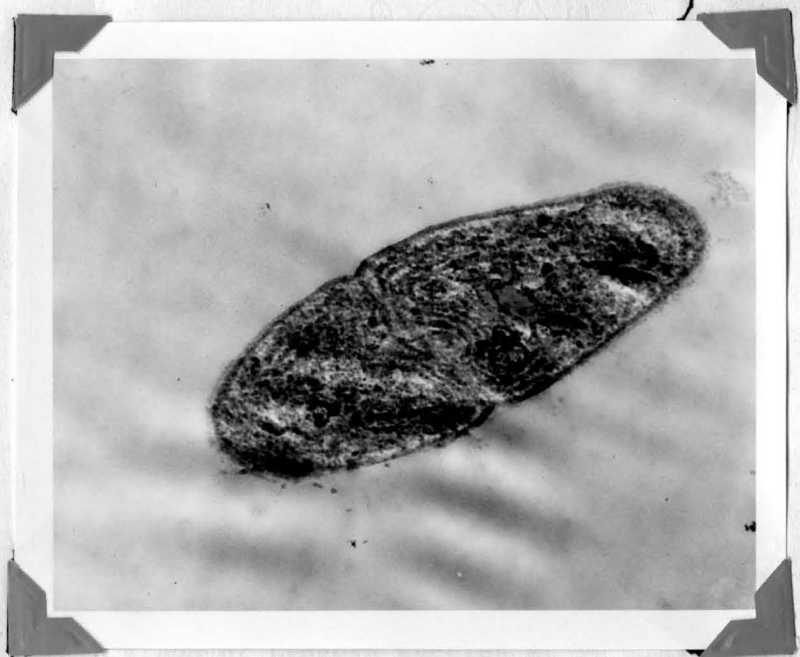


Fig. 23. Electron micrograph of Lyngbya species at 40 minutes after infection with blue-green algae virus. Healthy cells. Magnification X 79,000.





Fig. 24. Electron micrograph of Lyngbya species at 50 minutes after infection with blue-green algae virus. Healthy filament. Magnification X112,000.

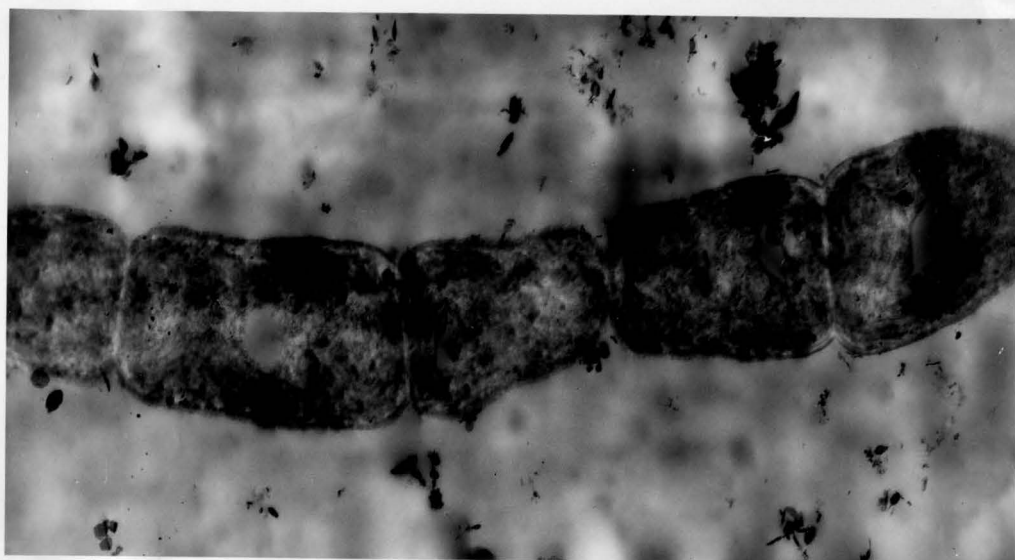


Fig. 25. Electron micrograph of Lyngbya species at 55 minutes after infection with blue-green algae virus. Healthy filament. Magnification X 75,000.

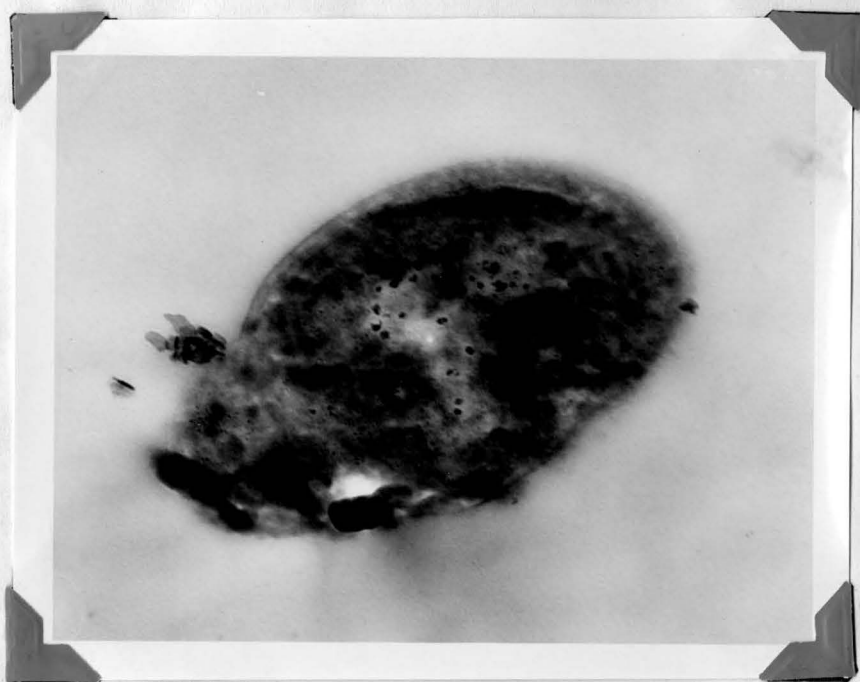


Fig. 26. Electron micrograph of Lyngbya species at 60 minutes after infection with blue-green algae virus. Infected filament. Magnification X 150,000.



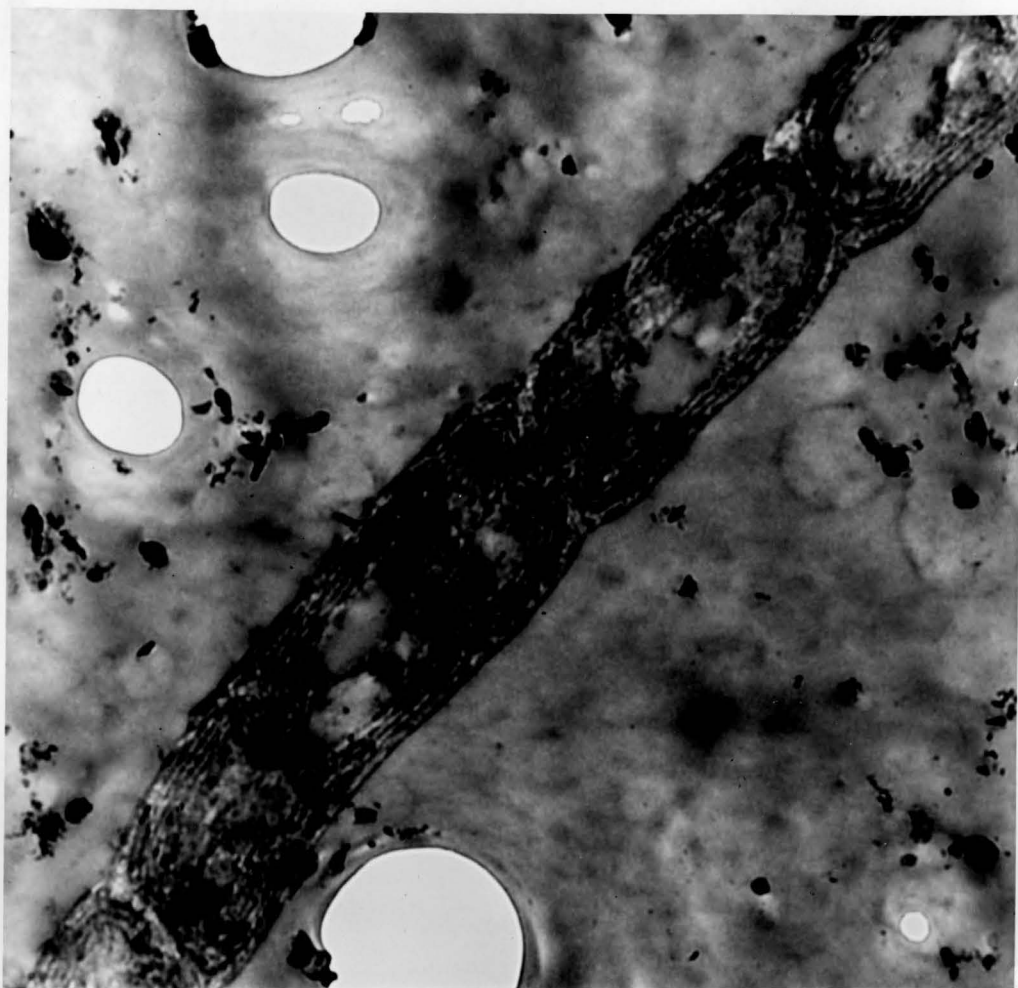


Fig. 27. Electron micrograph of Lyngbya species at 70 minutes after infection with blue-green algae virus. Infected filament. Magnification X 56,000.

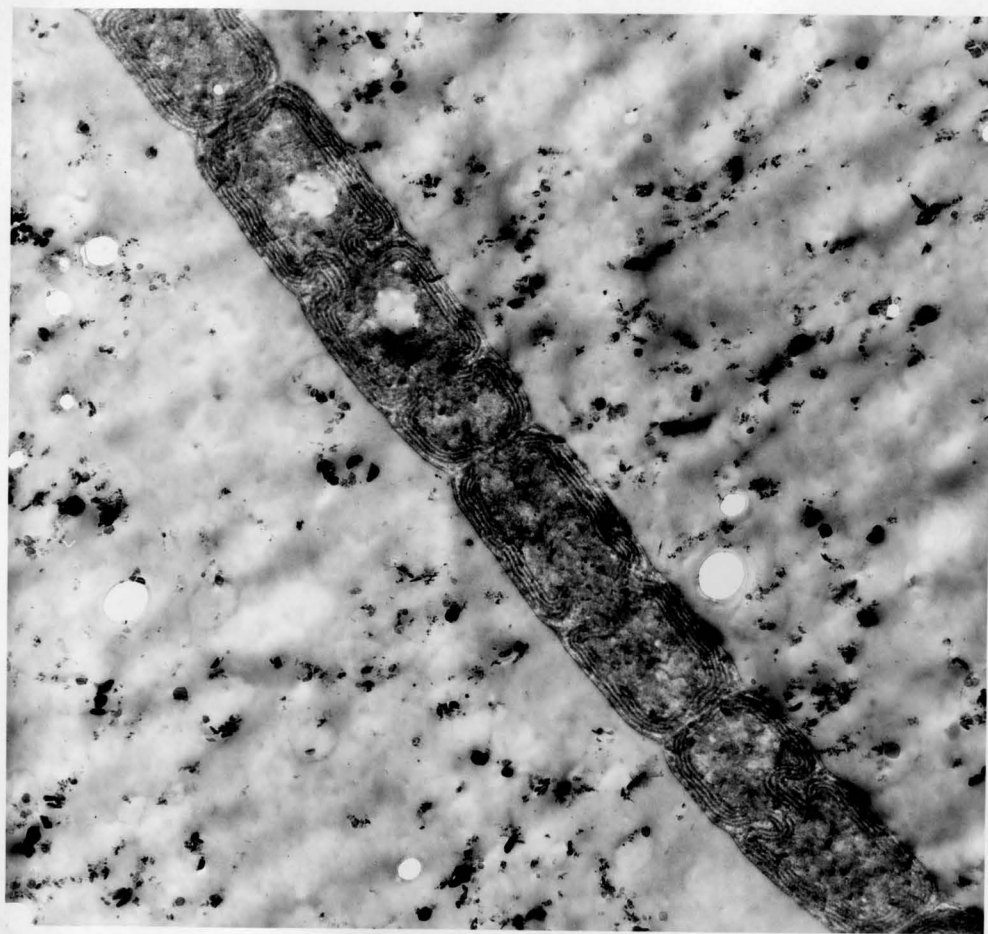


Fig. 28. Electron micrograph of Lyngbya species at 70 minutes after infection with blue-green algae virus. Infected cells. Magnification X 24,000.

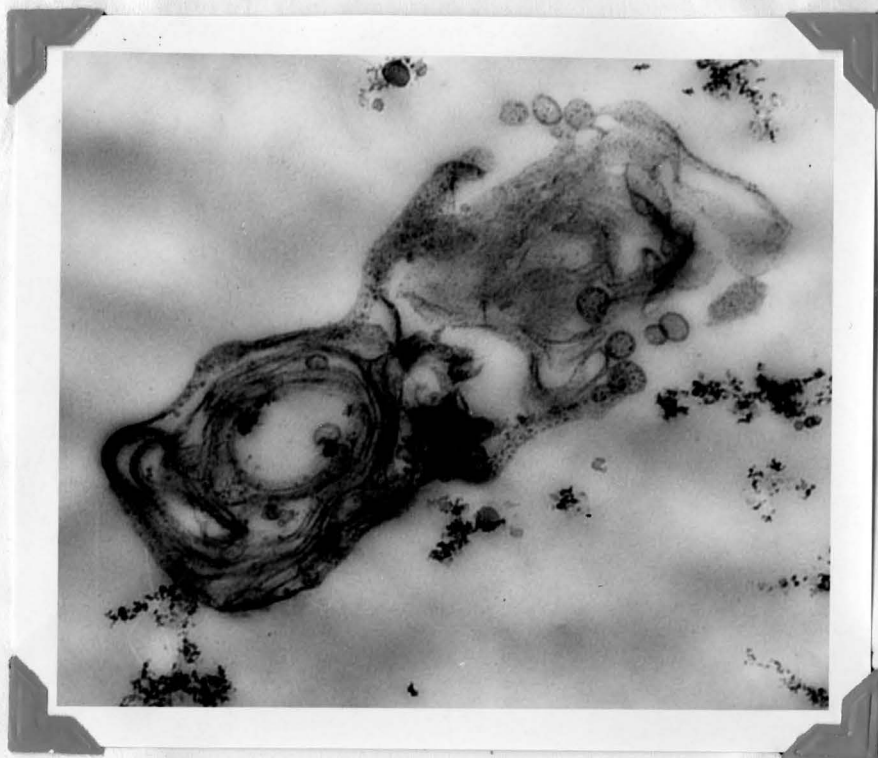


Fig. 29. Electron micrograph of Lyngbya species at 80 minutes after infection with blue-green algae virus. Lysed cells with virus particles. Magnification X 109,000.

Plectonema boryanum was the first species of the blue-green algae to be found susceptible to virus infection. Its morphology appears to be less complex than that observed in several other members of Cyanophyta. In the electron micrographs taken, the sheath was not evident in both Lyngbya species and Plectonema boryanum. It was perhaps due to its disintegration during fixation. The plasma membrane enclosing the internal cellular components was apparent. Longitudinal sections reveal the photosynthetic lamellae lying parallel to the cell walls forming a symmetrical pattern of four to five concentric rings. The lamellae appear to be composed of flattened cisternae or disks of variable length. Interlamellar matrix showed ribosome-like particles. Occasionally quite large and dense particles were noted which might correspond to alpha and beta granules as described by Pankratz and Bowen (1963). Uninfected cells manifested a diffuse distribution of nucleoplasm (figs. 1 and 18), as seen in other blue-green algae, with no limiting membrane defining the nuclear area in these cells. Such areas of relatively low density occur in a regular pattern in the central regions of the cells (fig. 20).

The cellular components, apart from those in the interlamellar matrix, include small dense granules, approximating the size and shape of ribosomes, occasional vacuole-like structures and large bodies of variable sizes and density (fig. 3). Similar bodies have been found by other workers as structured granules (Drews and Nicklowitz, 1956, 1957; Pankratz and Bowen, 1963), or polyhedral bodies (Jensen and Bowen, 1961) depending on the characteristics of size, shape, density position and staining quality. Other organelles found in higher algae, such as mitochondria and Golgi bodies are not found in blue-green algae.

It seems logical that the sheath and wall of the algae require abrasion

or chemical dissolution for entry by a virus. Schneider et al (1964) indicated a similarity in chemical composition of the cell walls of strains susceptible to virus and of bacterial cell walls. Their observations indicate that these algal strains are more closely related to bacteria than to higher plants.

Reorientation of the lamellae is found to be the most typical symptom of viral infection. The lamellae become increasingly less parallel and can be seen to pull completely away from the cell margins. These findings are in complete agreement with the findings of Smith et al (1966). Inside the cell the virus particles appeared to attach themselves to the photosynthetic lamellae and as virus particles form they remain attached to the lamellae even when the cells are completely lysed. As the infection advances in the intact cells some of the lamellae degenerate and are broken. In the final stages of infection (figs. 1 and 29) only a skeleton of the filament cell is left. These are remnants of photosynthetic lamellae, interspersed with virus particles still firmly attached to the lamellae.

The occurrence of slow infection in Lyngbya species as compared to Plectonema boryanum is attributable probably to the thickness and chemical composition of the sheath or other unknown internal genetic factors.

## SUMMARY AND CONCLUSIONS

The blue-green algae Plectonema boryanum and Lyngbya species were cultured on a synthetic liquid medium at 21°C under fluorescent light, intensity at 200 ft-c. Three week old cultures, 100 ml each in 300 ml Belco flasks were inoculated with 1 ml of partially purified blue-green algae virus.

From the inoculated cultures 20 ml samples were withdrawn at 5 minute intervals. Samples were centrifuged at 2000 RPM in a refrigerated RC B-2 centrifuge for five minutes. Pellets were fixed in a acrolein-glutaraldehyde-cacodylic buffer solution for one hour at room temperature. The samples were then postfixed in 2% osmium tetroxide at room temperature for one hour and then finally left overnight at 5°C in 0.5% uranyl acetate before dehydration. Sections were cut with a Porter-Blum ultramicrotome with a glass knife and were then stained first in uranyl acetate and finally counter stained in lead citrate. Electron micrographs were taken with a Hitachi model HU-11 electron microscope.

In the infected cells of algae photosynthetic lamellae invaginated. Virus particles were seen in contact with the cell wall and inside the cell attached to the photosynthetic lamellae. Here the particles remained attached after the cells were completely lysed. Virogenic stroma appeared after the infection process had started.

The time at which infection started was five minutes or earlier in Plectonema boryanum and twenty minutes in the case of Lyngbya species. Complete lysis of Lyngbya species occurred at 80 minutes after infection with blue-green algae virus.

LITERATURE CITED

- Bald, J. G., and R. A. Solberg. (1961). Apparent Release of Tobacco Mosaic Virus in Living Cells. *Nature* 190: 651-652.
- Burlew, J. S. (1953). Algal Culture, from Laboratory to Pilot Plant. Carnegie Institute of Wash. Publ. 600.
- Brown, Jr., R. M., K. M. Smith and P. L. Walne. (1966). Replication Cycle of The Blue-Green Algal Virus LPP-1. *Nature* 212: 729-730.
- Cochran G. W., A. S. Dhaliwal, G. W. Welkie, J. L. Chidester, M. H. Lee and B. K. Chandrasekhar. (1962). Biosynthesis of Infectious Tobacco Mosaic Virus Ribonucleic Acid in a Cell Free Medium. *Science* 138: 46-48.
- Cornuet, P. and S. Astier-Manifacier. (1962). Essais de Biosynthese Enzymatique in Vitro du Pouvoir Infectieux du Virus de la Mosaïque du Tabac. *Acad. des Sci. Compt. Rend.* 255: 3076-3078.
- Drews G. and W. Niklowitz. (1956). Beitrage zur Cytologie der Blaualgen. II. Zentroplasma und Granulare Einschlusse von Phormidium Uncinatum. *Arch. Mikrobiol.* 24: 147-162.
- Dhaliwal, A. S. and G. K. Dhaliwal. (1968). Purification of Blue-Green Algae Virus by Chromatographic Method. *Advancing Front. Plant Sci.* 21: 195-203.
- Engler, R., and G. Schramm. (1959). Infectious Ribonucleic Acid as Precursor of Tobacco Mosaic Virus. *Nature* 183: 1277-1279.
- Fraenkel-Conrat, H., B. Singer, and S. Valdee. (1958). The Mechanism of Plant Virus Infection. *Biochimica et Biophysica Acta* 29: 639-640.

Franklin R. E., D. L. D. Casper, and A. Klug. (1959). Plant Pathology--Problems and Progress. 1908-1958. University of Wisconsin Press, Madison.

Gierer, A. (1958). Structure and Biological Function of Ribonucleic Acid from Tobacco Mosaic Virus. Nature 179: 1297-1299.

Goldstein, D. A., I. J. Bendet, M. A. Lauffer and K. M. Smith. (1967). Some Biological and Physicochemical Properties of Blue-Green Algal Virus LPP-1. Virology 32: 601-613.

Hausers-Casterman, C., and R. Jeener (1957). An Initial Ribonuclease-Sensitive Phase in the Multiplication of Tobacco Mosaic Virus. Virology 3: 197-206.

Jensen, T. E., and C. C. Bowen. (1961). Organization of the Centro-Plasm in Nostoc Pruniforme. Iowa Acad. Sci. Proc. 68: 86-89.

Karasek, M., and G. A. Schramm. (1962). Ribonucleotide Polymerase from Tobacco Leaves and the Formation of Viral Ribonucleic Acid in Vitro. Biochem. and Biophys. Res. Commun. 9: 63-68.

Kassanis, B. (1960). Comparison of the Early Stages of Infection by Intact and Phenol-Disrupted Tobacco Necrosis Virus. Virology 10: 353-369.

Kim, Y. T., and S. G. Wildman. (1962). Synthesis of Infectious Tobacco Mosaic Virus RNA by Cell-Free Extracts from Tobacco Mosaic Virus Infected Tobacco Leaves. Biochem. and Biophys. Res. Commun. 8: 394-401.

Luftig, R., and R. Haselkorn. (1967). Morphology of a Virus of Blue-Green Algae and Properties of its Deoxyribonucleic Acid. J. Virology 1: 344-361.



- Luftig, R. and R. Haselkorn. (1968). Studies on the Structure of Blue-Green Algae Virus LPP-1. *Virology* 34: 664-674.
- Padan E., D. Ginzburg and M. Shilo. (1970). The Reproductive Cycle of Cyanophage LPP 1-G in Plectonema Boryanum and its Dependence on Photosynthetic and Respiratory Systems. *Virology* 40: 514-521.
- Pankratz, H. S. and C. C. Bowen. (1963). Cytology of Blue-Green Algae. I. The Cells of Symploca Muscorum. *Amer. J. Botany* 50: 387-399.
- Safferman, R. S. and M. E. Morris. (1963). Algal Virus: Isolation. *Science* 140: 679-680.
- Safferman R. S. and M. E. Morris. (1964). Growth Characteristics of the Blue-Green Algal Virus LPP-1. *J. Bact.* 88: 771-775.
- Safferman, R. S. and M. E. Morris. (1967). Observations on the Occurrence, Distribution, and Seasonal Incidence of Blue-Green Algal Viruses. *Appl. Microbiol.* 15: 1219-1222.
- Safferman, R. S., I. R. Schneider, R. L. Steere, M. E. Morris and T. O. Diener, (1969). Phycovirus SM-1: A Virus Infecting Unicellular Blue-Green Algae. *Virology* 37: 386-395.
- Schramm, G. and B. Rottger. (1959). Untersuchungen Uber das Tabakmosaikvirus mit Fluoreszierenden Antikörper. *Zeit. Naturforsch.* 144: 510-515.
- Schneider, J. R., T. O. Diener and R. S. Safferman. (1964). Blue-Green Algal Virus LPP-1. Purification and Partial Characterization. *Science* 144: 1127-1130.

Schwimmer, M., and D. Schwimmer. (1955). The Role of Algae in Plankton and Medicine. Grune and Stratton. New York.

Siegel A. W., W. Ginoza and S. G. Wildman. (1957). The Early Events of Infection with Tobacco Mosaic Virus Nucleic Acids. Virology 3: 554-559.

Singh, R. N. and P. K. Singh. (1967). Isolation of Cyanophages from India. Nature 216: 1020-1021.

Smith, K. M., R. M. Brown, Jr., D. A. Goldstein, P. O. Walne. (1966a). Culture Methods for the Blue-Green Alga Plectonema Boryanum and its Virus with an Electron Microscope Study of the Virus-Infected Cells. Virology 28: 580-591.

Smith, K. M., R. M. Brown, Jr., P. L. Walne, D. A. Goldstein. (1966b). Electron Microscopy of the Infection Process of the Blue-Green Algal Virus. Virology 30: 182-192.

Smith, K. M., R. M. Brown, Jr., P. L. Walne. (1967). Ultrastructural and Time-Lapse Studies on the Replication Cycle of the Blue-Green Algal Virus LPP-1. Virology 31: 329-337.

Wildman, S. G. (1959). In: Burnet and Stanley eds. The Viruses. 2, ch. I. Academic Press, New York.

APPROVAL SHEET

The thesis submitted by Thomas A. Rudd has been read and approved by the director of the thesis.

Furthermore, the final copies have been examined by the director and the signature which appears below verifies the fact that any necessary changes have been incorporated, and that the thesis is now given final approval.

The thesis is therefore accepted in partial fulfillment of the requirements for the degree of Master of Science.

May 22, 1972  
Date

A-S. Dhalwal  
Signature of Advisor